SERPENTS' VENOM:

ARTIFICIAL AND NATURAL IMMUNITY; ANTIDOTAL PROPERTIES OF THE BLOOD-SERUM OF IMMUNIZED ANIMALS AND OF VENOMOUS SERPENTS.

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On the Rendering of Animals Immune against the Venom of the Cobra and other Serpents; and on the Antidotal Properties of the Blood-Serum of the Immunized Animals. By Professor Thomas R. Fraser, M.D., F.R.S. (With a Diagram.)

(Read June 3, 1895.)

(*Abstract.*)

One of the most striking and interesting of the many traditions and current beliefs regarding venomous serpents is that a power may be acquired of freely handling them without injury, and even of sueeessfully resisting the poisonous effects of their bites.

The Psylli of Africa, the Marsi of Italy, the Gouni of India, and other aneient tribes and seets, were stated to have been immune against serpents' bites, and to have been able to exereise a remarkable influenee over even the most venomous of these animals; and these attributes have been explained on the supposition that serpents' blood was present in the veins of the members of these tribes and sects.

In more modern times, and, indeed, at the present day, the same belief is stated in the writings of travellers; and it has been expressed by poets and novelists, and among the latter, with a half-admitted eonviction of its reality, by Wendell Holmes, in his *Romance of Destiny.*

In "a new and accurate Deseription of the Coast of Guinea," published in 1705, by William Bosman, an aecount is given of the great "reverenee and respeet" of the negroes for snakes worshipped by them as gods; in eonneetion with whieh the following statements are made :—"But what is best of all, is, that these idolatrous snakes don't do the least misehief in the world to mankind; for, if by ehance in the dark one treads upon them, and they bite or sting him, it is not more prejudieial than the sting of millepedes. Wherefore, the natives would fain persuade us that it is good to be bitten or stung by these snakes, upon the plea that one is thereby seeured and proteeted from the sting of any poisonous snake. But here," he proeeeds to remark, "I am somewhat dubious, and should be loth

to venture on the credit of their asscrtions, beeause I have observed that the gods themselves are not proof against these venomous serpents, mueh less ean they proteet us against their bite."

Drummond Hay, in his work on Western Barbary, gives a deseription of the performances of four members of a seet of snake-eharmers, called the Eisowy (Aissaivi), who freely handled, and allowed themselves to be bitten by serpents proved to be venomous by a rapidly fatal experiment performed on a fowl. At the termination of the exhibition, the Eisowy, apparently as a usual performance, "eommenced eating or rather ehewing" a poisonous snake, "whieh, writhing with pain (to quote Mr Hay's words) bit him in the neek and hands until it was aetually destroyed by the Eisowy's teeth." He states that, on another oeeasion, at Tangier, a young Moor, who was witnessing the performances of a snake-eharmer, ridieuled his exhibition as a delusion, and having been dared by the Eisowy to toueh one of the serpents the lad did so, was bitten by one of them, and shortly afterwards expired. In conneetion with my subjeet, a speeial interest is attaehed to the account given by Mr Drummond Hay, and repeated in its main features by Quedenfeldt in the *Zeitschrift für Ethnologie* of 1886, of the origin of this Eisowy (Aissaivi) sect, and of the immunity whieh they elaim. The founder, Seedna Eiser, was being followed through the desert of Soos by a great multitude, who, beeoming hungry, elamoured for bread. On this, Seedna Eiser beeame enraged, and turning upon them he uttered a eommon Arabic eurse, "kool sim," whieh means "eat poison." So great was their faith in the teaehing of the saint, that they aeted upon the literal interpretation of his words, and thereafter ate venomous snakes and reptiles; and from that time they themselves and their deseendants have been immune against serpents' bites.

In the writings of many other travellers similar evidenees may be found of a belief in the possession of a power successfully to resist the poisonous effects of serpents' bites. The same belief presents itself in the eonviction, prevailing in several parts of the world, that a non-fatal bite by a poisonous serpent, provided marked symptoms have followed, confers protection against subsequent bites; and in the tales of the performanees by the snake-charmers of the present time. These performanees have been

graphically described, among others, by Hooker and Ball in their *Journal of a Tour in Marocco and the Great Atlas*, but only to be dismissed as impostures, rendered possible by the previous extraction of the poison-fangs, or by some other disabling operation. Although, possibly, the performances are at times, or even frequently, impostures, it almost appears as if this conclusion were arrived at more because of their improbability and their apparent defiance of knowledge regarding the effects of serpents' venom, than because of satisfactory or sufficient proof having been obtained of the conclusion. Some of the facts which I shall bring before the Society will, on the other hand, show that this conclusion can no longer be justified on the ground that the asserted facts imply impossibilities.

It may be instructive to associate with this belief in the possession, under certain conditions, by human beings of a power successfully to resist the poisonous effects of serpents' venom, and with the evidences in its support, the further belief that venomous serpents are themselves protected against the effects of bites inflicted upon them by individuals both of their own and of other species. On mere anatomical grounds it is difficult to understand how serpents could escape the absorption of their own venom through mucous surfaces, even admitting that absorption of venom does not occur in normal conditions of these surfaces. Venom must, however, be so frequently introduced into their bodies, in situations where absorption could not fail to occur, by the bites inflicted upon them by other serpents, that the conclusion seems inevitable that they possess some protective quality, without which, probably, no venomous serpents would now be in existence. Not only have many general observations been made in support of this belief, but it has been proved to be correct by direct experiments, such as those made by Fontana of Tuscany more than a century ago, and by Guyon, Laçerda, Waddell, Kaufmann, and Sir Joseph Fayrer.

This, and other evidence, pointing to the existence of protection against venom, not only in serpents themselves, but also, in certain exceptional circumstances, in human beings, several years ago originated a wish to further investigate the matter. It was obviously suggested that if protection occurs, it must be caused by

some direct result of the absorption of venom; and, therefore, that its existence could be proved or disproved by experiment. In the former event, the first steps would already have been taken to obtain, by further experiments, results likely to be of value in the treatment of poisoning by serpents' venom; and, indeed, likely to be of importance in even the wider field of general therapeutics.

With these objects, endeavours were made to collect a sufficient quantity of venom; but the collection has proceeded but slowly, and only after several years has a supply gradually been accumulated sufficiently large to render it probable that definite results would be obtained before the supply of venom had become exhausted in the experiments.

I received my first supply of cobra venom in 1879, from Surgeon-Colonel Moir, lately of Meerut, and afterwards—also in small quantities—from the late Dr Shortt of Madras, and from Sir Joseph Fayrer, the Thakore of Gondal, and Dr Phillips. Larger quantities were subsequently obtained from Surgeon-Captain French, and through the kind efforts of Sir William Mackinnon, Director-General of the Army Medical Department, from each of the Presidencies of India. Early in this year, an additional supply was received from Surgeon-Colonel Cunningham of Calcutta, and this gentleman has quite recently sent a further large quantity of several grammes of dry venom.

But, besides these specimens of the venom of the cobra of India, I have also been fortunate in obtaining specimens of venoms from other parts of the world.

From America, Dr Weir Mitchell of Philadelphia—whose work on the chemistry and physiology of serpents' venom constitutes the great advance of the century on the venom of viperine serpents—has supplied me with the venom of three species of rattlesnakes—viz., *Crotalus horridus*, *C. adamanteus*, and *C. durrisus*, and also with a specimen of the venom of the Copper Head (*Trigonocephalus contortrix*).

From Australia, Dr Thomas Bancroft, of Brisbane, has at various times sent specimens of the venoms of the black snake (*Pseudechis porphyriacus*), the brown snake (*Diemenia superciliosa*), and of a large unidentified snake of the Diamantina district of South Australia (probably a new species of Diemenia).

From Africa, the kindness of Mr Wm. Smith, a distinguished naturalist of Cape Town, of Dr Brock of the Orange Free States, and of Dr John Murray and Mr Van Putten of Cape Colony, has placed at my disposal small quantities of the venom of the puff adder (*Vipera arietans*), the night adder (*Aspidelaps lubricus*), the yellow cobra (*Naja haie*), and the "Ring Hals Slang" or "Rinkas" (*Sepedon hæmachates*); and Dr John Anderson, formerly Professor of Natural History at Calcutta, has, only last week, forwarded to me living specimens of the *Vipera cerastes*, to be followed by living specimens of the cobra, which his present connection with the zoology of Egypt has given him peculiar facilities to obtain.

In the meantime, however, further evidence has been obtained in support of the reality of the probabilities to which I have referred. Sewall, using the venom of the rattlesnake, Kanthack that of the cobra, and Kaufmann and Phisalix and Bertrand that of the viper, obtained experimental evidence of the possibility of producing by "inoculation" a certain slight degree of resistance against the toxic effects of these venoms. The relationship of such observations to the recent discoveries in connection with the toxines of Tetanus, Diphtheria and other diseases, could not long remain unrecognised. Dr Bancroft and others have recently suggested "that the blood of animals rendered immune to snake venom might be found of service as a remedy in snake-bite." Within the last few months, Phisalix and Bertrand have obtained experimental indications of the antidotal power of the blood-serum of animals immunized, but only to a low degree, against the venom of vipers; while Calmette, working in the Pasteur Institute of Paris, after several unsuccessful endeavours had led him to express the opinion that immunity against snake venom could not be produced, afterwards succeeded in obtaining evidence of its production, and of the power of the blood-serum to counteract the effects of venom.

In the case of many of the venoms which I have had the good fortune to obtain, the quantity at my disposal was not sufficient for experimental examination on the plan that seemed desirable, and, besides, the examination of each of them would require several months of work. In this, the first portion of the investigation, therefore, the venoms that have been used are only four in

number, those, namely, of the cobra of India (*Naja tripudians*), of the *Crotalus horridus* of America, of a large colubrine snake, probably a species of *Diemenia*, from South Australia, and of the *Sepedon hæmachates* of Africa. The venoms are therefore those of the most deadly of the poisonous serpents of Asia, America, Australia, and Africa, respectively; and further, they are representative of the chief differences that occur in the composition and action of venoms, for they are derived from members of the two great groups of the colubrine and viperine serpents.

My supply of cobra venom being much larger than that of any of the others, this venom was chiefly used in the experiments; and in all of those to be referred to to-night, the administration was effected by subcutaneous injection.

An essential preliminary to exact investigations with active substances must always be the determination of the activity of the substances. The only convenient method for doing this is to define the smallest dose capable of producing death for any given weight of animal—that is, the minimum-lethal dose. The venoms in their natural state are inconstant in activity, mainly because of variations in the quantity of the water which they contain. The cobra venom has, however, nearly always been received in the form of a dry solid; but when this was not so, it has been dried *in vacuo* over sulphuric acid.

Outside of India, there are few persons skilled in the hazardous task of taking venom directly from living serpents. Accordingly, with a few exceptions, the other venoms were not received in a pure form, but in the form of the dried venom glands. From these glands, however, the poisonous constituents may easily be extracted with water, and, on evaporating the solution over sulphuric acid, an active dry venom is obtained, containing, however, other substances besides those which are active. I am not in a position, therefore, to make any statement in regard to the relative activity of the different venoms. For the objects in view, what only is necessary is that the exact minimum-lethal dose should be known of each venom in the state in which it is used, whether it be pure or diluted with a certain small amount of inert matter.

Each of the four venoms was, however, found to be very active, but the cobra venom especially so,—a part of the difference between

its activity and that of the other venoms being, no doubt, due to the above circumstances.

Experiments were made with cobra-venom on several animals—as the guinea-pig, rabbit, white rat, cat, and the innocuous grass snake of Italy (*Tropedonotus natrix*). Very considerable differences were found to occur in the minimum-lethal dose for these animals. For the guinea-pig, the minimum-lethal dose per kilogramme was ·00018 grm.; for the rabbit, ·000245 grm.; for the white rat, ·00025 grm.; for the cat, somewhat less than ·005 grm.; and for the grass snake, the relatively large dose of ·03 grm.* Cobra venom thus takes a position among the most active of known substances, rivalling in its lethal power the most potent of the vegetable active principles, such as aconitine, strophanthin, or acokantherin.

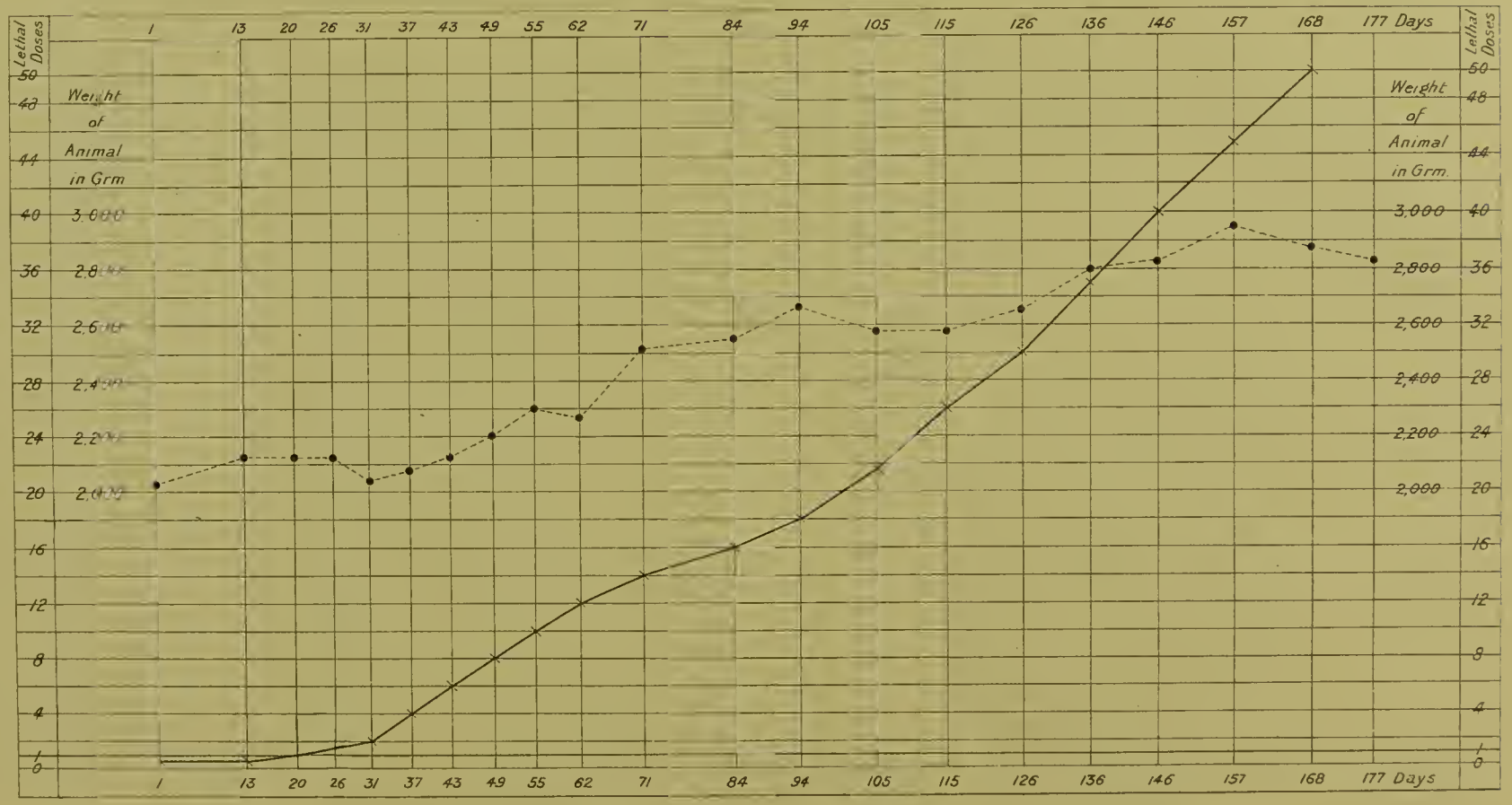
These facts having been ascertained, attempts were next made to render animals proof against lethal doses, by administering to them a succession of gradually increasing non-lethal doses. These were, for the first few doses, in some of the experiments, one-tenth of the minimum-lethal, in others one-fifth, in others one-half of the minimum-lethal, and in others almost as great as the minimum-lethal. At varying intervals, the doses were repeated, and by-and-by gradually increased, until the actual minimum-lethal had been attained. The subsequent doses, by gradual increments, exceeded the minimum-lethal, and after five or six times the minimum-lethal had been reached, it was found that the increments could be further increased, so that each became twice, four times, and latterly even five times the minimum-lethal, and still the animal suffered little, and, in many cases, no appreciable injury.

This brief statement, however, does not represent the experimental difficulties that were encountered. It describes the course of events in the altogether successful experiments. Non-success, however, was frequent, and many failures occurred before experience indicated the precautions and conditions that are necessary for success.

Serpents' venom exerts what may broadly be described as a duplex action. It produces unseen functional disturbances, and it

* Guinea-pig, nearly $\frac{1}{5}$th millig.
Rabbit, nearly $\frac{1}{4}$th millig.
White Rat, $\frac{1}{4}$th millig.
Kitten (6 weeks), 2 millig.
Cat, 5 millig.
Grass Snake, 3 centig.

IMMUNIZATION OF A RABBIT AGAINST 50 TIMES THE MINIMUM-LETHAL DOSE OF COBRA VENOM.



A. RITCHIE & SON EDIN.ᴿᵍ

THE CROSSES CONNECTED BY THE CONTINUOUS LINE REPRESENT ADMINISTRATIONS OF VENOM.

THE DOTS CONNECTED BY THE INTERRUPTED LINE REPRESENT THE WEIGHTS OF THE ANIMAL.

also produees visible ehanges. Thc latter are of a highly irritative eharactcr, eausing intense visceral eongestions in the lungs, kidneys and other organs, and, when given by subeutaneous injection, on all the struetures of the skin and subjacent parts. There are apparently also some definite ehanges produeed in the blood, with regard to which several important faets have been diseovered by Dr Martin of the University of Sydney. Irritative effeets are obviously produced by eobra venom, even in non-lethal doses, and with greatly increased virulenee by doses that exceed the minimum-lethal; but, in respect to this aetion, the other three venoms used are greatly more aetive than the venom of the cobra. Evidenee was obtained to indieate that in the proeess of immunization, a diminution oeeurs in the intensity of these local aetions; but this diminution does not proeeed so rapidly as that in the unseen funetional or other ehanges whieh are the more direet eauses of death; and, further, the local irritative ehanges, after having been produeed, are slower to disappear than the unseen funetional disturbanees. Until these faets had been appreciated, and, indeed, even with the adoption of preeautions suggested by them, frequent failures oeeurred. The apparently eontradictory results, aeeordingly, were obtained of the produetion, by gradually inereasing doses, on the one hand, of a proteetion against quantities mueh above the minimum-lethal, so perfeet that no apparent injury was eaused; and, on the other hand, of an intoleranee so deeided that death was produeed by the last of a suecession of gradually increasing doses, no one of which was so large as the minimum-lethal. The latter unfortunate event was frequently displayed in guinea-pigs, and attempts to earry immunization in them to a high point were found to be extremely diffieult.

Notwithstanding these diffieulties, however, sueh gratifying results have been obtained as that rabbits eould at last reeeive, by subcutaneous injeetion, so much as ten, twenty, thirty, and even the remarkable quantity of fifty times the minimum-lethal dose, without manifesting any obvious symptoms of poisoning. (See Diagram.)

Almost the only observable phenomena were a rise in the body temperaturc, whieh eontinued for a few hours after the injeetion, and which contrasts with the fall that oeeurs, after the administration of even non-lethal doses, in non-protected animals; and a loss

of appetite, which usually, though not invariably occurred, and was probably the cause of a temporary slight fall in weight during the day or two days succeeding each injection. On the other hand, during the process of successful immunization, the animals increased in weight, they fed well, and appeared to acquire increased vigour and liveliness. This has been frequently exemplified in the smaller animals, such as rabbits; and also, very conspicuously, in an aged and previously sedate horse, which, in the process of immunization, has now received eleven times the estimated minimum-lethal dose.

It is marvellous to observe these evidences of the absence of injurious effects, and even of the production of benefit in an animal which, for instance, has received in one single dose a quantity of venom sufficient to kill, in less than six hours, fifty animals of the same weight, and, in the course of five or six months, a total quantity of venom sufficient to destroy the lives of 370 animals of the same species and weight. There are few facts in the whole range of biology more calculated to arrest the attention or produce astonishment in the mind of the observer!

With the cobra venom, I have also immunized cats, both by subcutaneous and by stomach administration; but the significance of the latter method of administration must be reserved for a future communication. As I have stated, a horse is also being immunized; and I have to express my obligations to Principal Williams and Professor W. Owen Williams, for granting me the accommodation of their establishment, and to Mr Davis, M.R.C.V.S., also of the New Veterinary College, for much valuable assistance.

Following the same plan of research with the three other venoms, it was found that the minimum-lethal dose per kilogramme for rabbits of the Diamantina venom is ·0015 grm.; of the venom of *Sepedon hæmachates*, ·0025 grm.; and of the venom of Crotalus ·004 grm.* The Crotalus venom is, in its purity, altogether comparable with the cobra venom; and the determinations, therefore, show that cobra venom is sixteen times more powerful than Crotalus or rattlesnake venom. This venom, as well as the two others, however, much exceed cobra venom in the intensity of their local

* Diamantina venom, 1½ milligramme.
Sepedon hæmachates, 2½ ,,
Crotalus horridus, 4 ,,

action. When death is produced by Crotalus venom, the subcutaneous tissues become extensively infiltrated with a large quantity of blood and of blood-stained serum, the underlying muscles are reduced to an almost pulpy blood-stained substance, and post-mortem decomposition occurs very soon after death. Similar changes in the subcutaneous tissues, but to a rather less degree, are caused by the Diamantina venom, and in addition, hæmaturia, or more probably hæmoglobinuria, was invariably produced by lethal and even by large non-lethal doses. I mention these circumstances to indicate the perfection of the protection which is produced by the administration of successive gradually increasing doses; for they can be so adjusted that a dose of each venom, even six times larger than the minimum-lethal, may be administered without producing more than an inconsiderable and often scarcely observable degree of local destructive effect.

In the meantime, the process of protection against the latter venoms has not advanced further than six times the minimum-lethal dose. This, however, has been sufficient to allow experiments to be made by which it has been demonstrated that when an animal has acquired a resistant power over more than the minimum-lethal dose of one venom, that animal is also able successfully to resist the lethal action of a dose above the minimum-lethal of other venoms. To a rabbit protected against cobra venom, a dose above the minimum-lethal of Sepedon venom has been administered; to rabbits protected against Crotalus venom, doses above the minimum-lethal of Diamantina and of cobra venoms have been given; to rabbits protected against the Diamantina venom, doses above the minimum-lethal of Crotalus and Sepedon venoms have been given, and in each case the animal has recovered, and but few symptoms of injury were produced. At the same time, in other experiments, evidence was obtained that animals protected against a given venom are capable of resisting the toxic effect of that venom more effectually than the toxic effects of other venoms.

My experiments have not yet proceeded sufficiently far to show for what length of time the protection conferred by any final lethal dose may last. I propose to make some experiments which will give definite information in regard to this point, which may possibly lead to practical applications. It has incidentally been discovered,

however, that protection lasts for at least a considerable period of time, even when the last protective dose has not been a large one. For example, to a rabbit which had last received twice the minimum-lethal dose of Crotalus venom, the same dose was administered twenty days subsequently, and it altogether failed to produce any toxic symptoms.

Before passing to the next part of my communication, it may be stated that as yet no sufficient data have been obtained for affording an explanation of these remarkable facts. It is obvious that the blood of protected animals must contain some substance or substances which are not present in the non-protected animals, by which the lethal and toxic effects of venoms are prevented. I have observed that when the blood-serum of protected animals is added to a solution of venom, a distinctly observable reaction occurs, and this reaction may be of significance when considered along with circumstances, which will be stated in the remaining part of this communication, and especially with the circumstance that the blood-serum itself possesses but little physiological activity. This protective substance may be produced in the body by the influence of the venom, but it is also conceivable that the substance is actually a part of the venom itself, which gradually accumulates under repeated administrations, whereas the lethal and toxic constituents of the venom are more rapidly destroyed or eliminated.

Having thus succeeded in producing a high degree of protection in animals against the toxic effects of serpents' venom, the blood-serum of these animals was, in the next place, collected for the purpose of testing its antidotal properties. In this portion of the investigation, the method followed was essentially the same as that described in a communication made by me to this Society in 1871, on "The Antagonism between the Actions of Physostigma and Atropia," as it appeared to be the most direct method for obtaining accurate knowledge of the value of an antidote.

A few preliminary experiments were early made with the serum of animals in whom the protection had not been carried to a high degree, and they were sufficient to show that antidotal properties are possessed even by this serum. It soon became apparent that in order to obtain some reasonable approximation to constancy in the conditions of the experiments, it was necessary

that the serum should be in such a state that it would remain unchanged during at least several weeks. It was found that this could be insured without any appreciable loss of antidotal power by drying the freshly separated serum in the receiver of an air-pump, over sulphuric acid, after it had been passed through a Chamberland's filter. A perfectly dry and easily pulverisable solid was thus obtained, which may be kept unchanged for probably an indefinite time, and from which a normal serum can readily be prepared as required, by merely dissolving a definite quantity of the dry serum in a definite quantity of water.

To this serum, whether in the dry form or in solution, it would be convenient to apply the name "*antivenene*."

The experiments now to be described were made with antivenene derived from the mixed serum of three rabbits, which had last received a dose of cobra venom equivalent to thirty times the minimum-lethal. I avoid the expression "immunized against" thirty times the minimum-lethal dose, for, as a matter of fact, an animal is always protected, or immunized, against a dose considerably above the last which it had received.

The experiments were so planned as to obtain, in three or four different conditions, as exact a definition as possible of the antidotal power of the antivenene. In the meantime, four series of experiments have been undertaken on rabbits. In one series, the venom was mixed outside of the body with the antivenene, and immediately thereafter the mixture was injected under the skin of the animal; in the second series, the venom and antivenene were almost simultaneously injected into opposite sides of the body; in the third series, the antivenene was injected some considerable time before the venom; and in the fourth series, the venom was first injected, and thirty minutes afterwards the antivenene. In the experiments of the third and fourth series, also, the venom and antivenene were injected under the skin of opposite sides of the body.

All, or nearly all, the experiments required to define the exact quantity of antivenene that is sufficient to prevent death from different lethal doses of venom have as yet been made only in the first and fourth of these series. They are, however, in some respects the most important of the series: as the conditions for

exactitude in simultaneous administration are perfectly obtained in the first series, and it, therefore, should constitute the basis for comparison between antivenenes derived from different sources; and as upon the results of the fourth series must depend the actual practical application of antivenene to the treatment of poisoning by serpents' bites.

In the experiments of the *first series*, the doses of cobra venom administered were the minimum-lethal, twice the minimum-lethal, thrice the minimum lethal, and four times the minimum-lethal. In the case of each dose, experiments were made with different quantities of antivenene so as to determine the smallest quantity required to prevent death. In order to render it certain, in this and the other series, that a lethal dose was always administered in the experiments with the so-called minimum-lethal, the minimum-lethal indicated by the previous experiments was not used, but instead of it a slightly larger dose (·00026 instead of ·000245 grm. per kilogramme).

When this certainly lethal dose, capable of causing death in five or six hours, was mixed with antivenene and the mixture then injected under the skin, it was found that so small quantities of antivenene were sufficient to prevent death, as ·5 c.c., ·25, ·1, ·05, ·02, ·01, ·005, ·004 c.c. ($\frac{1}{2}$, $\frac{1}{5}$, $\frac{1}{10}$, $\frac{1}{20}$, $\frac{1}{100}$, $\frac{1}{200}$, $\frac{1}{250}$ of a c.c.) for each kilogramme of the weight of the animal. With ·0025 c.c. ($\frac{1}{400}$), however, the animal died. The antivenene was therefore found to be so powerful as an antidote, in the conditions of these experiments, that even the $\frac{1}{250}$ part of a cubic centimetre, equivalent to about one $\frac{1}{15}$ part of a minim, acted as an efficient antidote. Even with the smaller of these doses of antivenene, there was almost no symptom of poisoning produced. In the experiments of this series with twice the minimum-lethal dose, recovery occurred when the doses of antivenene were ·75 c.c., ·7 c.c. and ·6 c.c. per kilogram, but ·5 c.c. per kilogram failed to prevent death. In the experiments with thrice the minimum-lethal dose of venom (a dose capable of producing death in less than two hours), recovery occurred when the doses of antivenene were 1·5 c.c. and 1 c.c., but death occurred with ·8 c.c. And even the enormous dose of four times the minimum-lethal failed to produce death, or indeed any observable disturbance, when it had previously been mixed with 2 c.c. of antivenene for each kilogramme of animal.

In the *second* series, experiments have been made only with twice the minimum-lethal dose of venom. When this dose was injected into the subcutaneous tissue of one side of the body, and immediately thereafter a dose of antivenene, it was found that doses of 1 c.c., 2 c.c. and 3 c.c. per kilogramme failed to prevent death, but that 4 c.c. and 5 c.c. per kilogramme were able to do so.

In the *third* series, the experiments have as yet been made with only the minimum-lethal of cobra venom, and they show that ·4 c.c. per kilogramme of this antivenene is able to prevent death, when given thirty minutes before the venom.

In the *fourth* series, where the results are likely to give the clearest indications of the antidotal value of antivenene, it was found that recovery occurred in the experiments in which 1·5 c.c., 1 c.c., and ·8 c.c. per kilogramme of antivenene were injected *thirty minutes after* a *certain* minimum-lethal dose of venom, but that the antivenene was insufficient in quantity to prevent death when ·75 c.c. per kilogramme or less was administered. In this series, further, it was found that 5 c.c. per kilogramme of antivenene was a sufficient dose to prevent death after twice the minimum-lethal dose of venom; but that 2 c.c., 2·5 c.c., 3 c.c., and 4 c.c. per kilogramme were insufficient.

The experiments of this series are especially interesting, as nearly all the animals showed symptoms of poisoning before the antivenene had been administered. Even in the fatal experiments, the duration of life was greatly prolonged by the administration of antivenene; and it is probable that in many instances a second injection of antivenene, made half-an-hour or an hour after the first, would have prevented death.

It has thus been established, on the clearest evidence, that the blood-serum (antivenene) of animals protected against large lethal doses of venom is able, in varying conditions of administration, perfectly to prevent lethal doses of the venom of the most poisonous of serpents from producing death in non-protected animals.

In order to obtain some evidence bearing on the question as to whether the more powerful antivenene is produced by the long continued administration of small non-lethal doses of venom, or by the administration of doses gradually increasing until a large lethal dose is reached, a few experiments were made with the serum of a rabbit

which had received one-tenth part of the minimum-lethal dose nearly every two days during a period of three months and one week, and also of one which had received the one-fourth part of the minimum-lethal dose nearly every four days during a period of three months and three weeks. I did not find that the antidotal power of the antivenenes obtained from these animals was great, or nearly so effective as the antivenenes obtained from animals which had finally received a dose much in excess of the minimum-lethal. When mixed with venom and then injected, 3 c.c. per kilogramme of these antivenenes were insufficient to prevent death from somewhat more than the minimum-lethal dose of venom, but 5 c.c. per kilogramme were sufficient to do so.

I have also administered 1·5 c.c. per kilogramme of cobra antivenene thirty minutes after a dose one-twelfth larger than the minimum lethal of the venoms, respectively, of the *Sepedon hæmachates*, the *Crotalus horridus*, and the *Diamantina* serpent; and the rabbits experimented on have recovered. This successful result is all the more remarkable when the intensely destructive local effects of each, but especially of two, of these venoms is recollected.

The experiments establishing, and to some extent defining, the antidotal power of cobra venom, further, have been made on animals peculiarly susceptible to the poisonous action of serpents' venom, a circumstance of importance in considering the probable value of the antivenene when used as an antidote in the treatment of animals of less susceptibility, among whom there appears to be sufficient evidence to place human beings. The minimum-lethal dose for man probably approximates that of the cat, rather than that of vegetable feeders such as the rabbit, guinea-pig, and white rat.

It is also to be remembered that, in the meantime, the experiments have been restricted to a definition of the antidotal power in certain rigidly adhered to conditions, which were not always the most favourable for the mere prevention of death. Indications have indeed been obtained which render it highly probable that death may be prevented from occurring more certainly by several administrations, rather than by one administration of antivenene, and also by the introduction of the antivenene into the same parts as the venom, rather than into distant parts.

It would be important also to increase the number of the experi-

ments with the larger of the lethal doses of venom as yet administered, and, it may be, to employ still larger doses; although, for practical application, the larger of the doses that have already been used, as they produce death in about an hour, need not be increased.

To these purposes I hope to apply the antivenene soon to be prepared from the rabbits which have already received fifty times the minimum-lethal dose of venom.

For the actual application of the antivenene to the treatment of snake-poisoning in man, an endeavour is being made to obtain the large quantity that is requisite, from a horse now receiving considerable lethal doses of cobra venom. From this source, also, it is hoped that a sufficient quantity will be obtained to allow of the examination of the chemical properties of the antivenene to be continued, with the object of discovering the constituent or constituents by which the antidotal effects are produced. If the isolation of the antidotal constituent or constituents can be effected, an antivenene of greatly increased power will be obtained, and the range of efficient application will be increased. For these objects, however, it will be necessary to administer to the horse much larger doses than it has yet received; and the chief difficulty in doing this is to obtain a sufficient supply of cobra venom. By the great kindness of Surgeon-Colonel Cunningham, 9 grms. of dry venom have already been obtained, but in order to carry the protection to fifty times the minimum-lethal dose, other 30 grms. would be required. I have reason to hope that the India Office will succeed im making arrangements for procuring even this large quantity.

The subject is one of practical importance to India, where the destruction of human life by venomous serpents is represented by an annual mortality of 20,000, and where the failure of all methods of treatment* has led to the introduction of a system of extermination of venomous serpents—apparently futile in its results—in the carrying out of which large sums of money have been expended.

In considering the probabilities of success by antivenene treat-

* "After long and repeated observation in India, and subsequently in England, I am forced to the conclusion that all the remedies hitherto regarded as antidotes are absolutely without any specific effect on the condition produced by the poison."—Sir Joseph Fayrer *On the Nature of Snake Poison.*

ment, it is also to be recollected that antivenene can be obtained even more powerful than that which was used in the experiments which have been described; and that, judging from the statistics of Fayrer and Wall, in 75 per cent. of fatal cases in man death does not occur until from three to twenty-four hours after the infliction of the bite. This latter fact appears to indicate that in the great majority of the fatal cases the dose of venom does not much exceed the actual minimum-lethal; and, therefore, is not so large as the doses whose lethal action has been prevented from occurring in the experiments that have been described; in which, further, the conditions for success in preventing death were not the most favourable that could have been adopted.

It appears to me, however, that an interest and importance as great as can be derived from this practical application of the facts which I have brought before the Society, are to be found in their relation to the cause and treatment of many of the most fatal of diseases—those, namely, which are produced by organisms that have found their way into the body. The evidence in favour of the curative value of the antitoxines derived from animals immunized against the toxines of these diseases, seems to receive an additional confirmation from these facts. They also bring distinctly before us the circumstance that there are limits to this curative power, dependent on the dose of the toxine to be counteracted, on the special antidotal activity of the antitoxine that is used, and on the duration of the time during which the toxine has had an opportunity of exerting its poisonous action before the antitoxine is administered. If these and other conditions interfering with successful treatment are not determined and recognised, unmerited discredit is likely to be attached to remedies which alone of all remedies may be capable of preventing death in these diseases, by counteracting the effects of minimum-lethal and larger doses of the toxine.

Further Observations on Antivenene, and on the Production of Immunity against Serpents' Venom; with an Account of the Antidotal Properties of the Blood-Serum of Venomous Serpents. By Professor Fraser, M.D., F.R.S.

(Read July 15, 1895.)

(*Abstract.*)

At the time when my former paper was communicated to the Society, I was engaged in investigating several subjects closely related to those dealt with in that paper, but in regard to which the experimental work had not advanced sufficiently to allow definite statements to be made.

I propose, to-night, to make some statements on these subjects.

Antivenene of Rabbits protected against fifty times the minimum-lethal dose.—A description has already been given of the steps by which protection against fifty times the minimum-lethal dose of cobra venom had been produced in rabbits. The antidotal power of the antivenene derived from these rabbits has now been examined. When this antivenene was mixed with twice the minimum-lethal dose of cobra venom and the mixture injected under the skin of a rabbit, it was found that recovery occurred if the dose of antivenene was ·7 c.c. or ·6 c.c., but that the animal died if the dose was ·5 c.c. or ·4 c.c. per kilogramme. As ·65 c.c. per kilogramme of the antivenene derived from rabbits which had last received thirty times the minimum-lethal dose is able to prevent death when mixed with the same lethal dose of venom, this result is an unexpected one. It appears to show that the blood-serum of a rabbit which had last received thirty times the minimum-lethal dose of venom is almost as powerful as an antidote as the blood-serum of a rabbit which had last received fifty times the minimum-lethal dose. If this be the case, it is suggested that for any given species of animal there is a maximum limit to the quantity of antivenene which can be produced or retained in the blood, and that, in the case of the rabbit, this maximum limit is reached when the dose is thirty times the minimum-lethal or even somewhat less. It is also suggested that this maximum-limit is reached before the maximum protection of the animal has been produced; for, undoubtedly, an

animal which had last received fifty times the minimum-lethal dose will survive a larger subsequent dose of venom than an animal which had last received only thirty times the minimum-lethal dose. It is probable, therefore, that protection depends not only on the presence in the body of an antidotal substance, but also on a modification in the reaction of the tissues, produced by frequently repeated administrations, which lessen the susceptibility of the tissues to the injurious action of the venom.

Antivenene derived from the Horse.—Since my former communication, also, the blood-serum of the horse, then referred to, has been examined. The process of protection had been begun in February with one-fifth the minimum-lethal dose, estimated from the results obtained in other herbivorous animals. This dose was repeated in seven days, and again in five days. One-third the estimated minimum-lethal dose was next administered, then, on two occasions, one-half, then three-fourths, and then the actual minimum-lethal dose. By successive increments, the subcutaneous injections were continued until fifteen times the minimum-lethal was administered, four months and a half after the protecting process had been commenced.

Distinct general disturbance, including a rise of temperature, was produced by the earlier doses. The later and larger doses, however, have produced almost no general reaction, although both the earlier and the later doses have caused considerable local effects, and, conspicuously, subcutaneous œdema and necrosis of portions of the skin.

Ten days after fifteen times the estimated minimum-lethal dose had been administered, blood was taken, with careful antiseptic precautions, from the left jugular vein, and a considerable quantity of serum has thus been obtained. A small portion of this serum was preserved in the liquid state, but the greater part was dried *in vacuo* over sulphuric acid. It yielded 11·5 per cent. of solids in the form of a brittle substance, which was easily broken into bright, transparent, orange-yellow fragments.

The antidotal properties of this serum have been examined in two series of experiments. In the first, the serum, or antivenene, was mixed with cobra venom outside of the body; and in the second, cobra venom was injected thirty minutes before the serum.

In the former series of experiments, it was found that ·005 c.c.,

·004 c.c., ·003 c.c., ·002 c.c., and ·001 c.c. per kilogramme were each sufficient to prevent death from somewhat more than a minimum-lethal dose of venom, but that ·0005 c.c. ($\frac{1}{2000}$) was insufficient.

As the antivenene obtained from protected rabbits which had last received thirty times the minimum-lethal dose failed to prevent death, in the same conditions of experiment, when its dose was ·0025 c.c. ($\frac{1}{400}$th of a c.c.), the antivenene obtained from the horse is about twice as powerful as an antidote as the antivenene of rabbits protected against thirty and even fifty times the minimum-lethal dose.

When given thirty minutes after the same lethal dose of venom, this serum, further, was able to prevent death when the quantity injected was only ·5 c.c. per kilo.

In considering these results it must be recollected that the minimum-lethal dose of venom for horses has not yet been defined, and that no other data are available for forming an estimate than those derived from the determinations described in the former communication, which have been made in a few herbivorous animals. The dose last given to the horse may, therefore, have been considerably more than fifteen times the minimum-lethal dose. On the other hand, it may be the case that the maximum production or retention of antivenene occurs in the horse and other herbivorous animals with fifteen times the minimum-lethal dose, or, to use a chemical phrase, that with this dose the saturation-point of the blood has been reached.

Several interesting and practically important subjects for investigation are thus suggested, with regard to which information is likely to be obtained by an examination of the antivenene of the horse now undergoing protection, after the administration of the larger doses of venom which it is intended should be given.

Although it is certainly desirable that a still more powerful antivenene should be obtained, the antivenene already obtained is of sufficient antidotal power to be applied to the treatment of snake-bite in man; and I propose to send the greater part of it to India for this purpose.

For practical use, it is obvious that the antivenene in the dry state has advantages over a liquid preparation—in respect, for example, to portability, resistance to decomposition, and facility of subdivision into doses.

Some of these advantages are apparent when the specimens, now exhibited, of 15 c.c. of liquid antivenene are compared with the specimens of dry antivenene representing 15 c.c. of serum.

The facts which I had previously communicated to the Society show that the dry antivenene retains the original antidotal power of the liquid serum.

With this antivenene I have also made an experiment which illustrates its value when used as an antidote in actual practice, rather than when used merely for the purpose of defining its antidotal power in the rigidly adhered-to conditions of the experiments which have been described. ·5 c.c. per kilogramme having been found to be about the smallest quantity that can prevent death when given thirty minutes after rather more than the minimum-lethal dose of venom, this dose of venom was administered to a rabbit, and thirty minutes afterwards the insufficient dose of ·4 c.c. per kilogramme of antivenene. In three hours, the animal was lying extended with the head resting on the floor, limp and unable to stand; the respirations were infrequent and shallow; the cardiac action was feeble and irregular; and rattling sounds were being produced in the throat, from the excessive salivary and bronchial secretions always caused by toxic doses of cobra venom. A second dose, consisting of ·6 c.c. of antivenene, was now injected under the skin; and very soon a marked improvement occurred in the condition of the animal, the respirations becoming deeper, and the cardiac action stronger and more rapid, and without irregularity. An hour subsequently, a third dose of antivenene, consisting of ·5 c.c. per kilogramme, was injected; and further improvement was produced, so that all toxic symptoms soon disappeared, and the animal was restored to a nearly normal state, from which no relapse occurred until perfect recovery had become established.

Influence of Diet in modifying the Minimum-Lethal Dose.—I have already drawn attention to the remarkable difference in the minimum-lethal dose of venom for herbivorous as contrasted with carnivorous animals. If this difference be due, in any important degree, to the effects, transmitted and individual, of the special diet of each of these two groups of animals, it seemed probable that the minimum-lethal dose might be modified by changing the diet of any animal in whom this could be done

without much deterioration of health; for example, by restricting the diet of a herbivorous animal to animal food.

A number of young white rats, accordingly, were put on an animal dietary, as soon as they had been weaned; and, with the slight addition of a little vegetable food once or twice a week, found necessary to maintain them in fairly good health, this dietary was continued for seven weeks. To one of the rats, a dose of cobra venom one-and-a-half times greater than the minimum-lethal was then administered by subcutaneous injection, and, although marked symptoms of poisoning were produced, the rat recovered. Two weeks subsequently, the animal dietary having been continued, another of these white rats received twice the minimum-lethal dose, and it also recovered after a temporary illness. The experiments could not be carried further, as the other members of this family had fallen into bad health, and one after the other had died before this time.

In animals whose progenitors had subsisted mainly upon a vegetable diet, the conversion of the diet into that of carnivorous animals is, therefore, alone sufficient to reduce the vulnerability to venom, and to cause, in this respect, an approximation to the resistance of a carnivorous animal.

This fact appears to indicate that the toxic effects of serpent's venom are dependent to a large extent upon an influence on the blood, an influence as yet only partially and imperfectly recognised.

Protection produced by Stomach Administration.—In the experiments which I have hitherto described, and, indeed, apparently in all others made in this new subject of Serum Therapeutics, protection has been produced by the subcutaneous or, less frequently, the intra-venous injection of the venom or other toxic substance.

These methods of administration are attended with inconveniences, which, it seemed possible, might be avoided were the toxic substance introduced into the stomach or other part of the alimentary canal. No doubt, the probability of thus producing protection is opposed by the fact, recognised even at the time of Celsus, and corroborated by such modern observers as Laçerda, Weir Mitchell, Fayrer and Brunton, and Calmette, that serpents' venom is either altogether inert, or nearly so, when it is introduced into the stomach or any other part of the alimentary canal.

Even assuming that venom so introduced is inert, or nearly so, as a poison, it does not necessarily follow that it is incapable of producing protection; for this protection is, in part at least, dependent on the presence in the blood of a substance or substances which possess no distinct toxic action, and which may therefore be present in the blood as a result of the administration of venom, even although the venom did not produce any evident poisonous symptoms.

In order to obtain some evidence on this subject, the process for producing protection already described was applied to a cat, with the modification that the doses of venom were introduced into the stomach instead of being injected under the skin.

Taking as a basis the minimum-lethal dose by the latter method of administration, the cat received at intervals of from two to five days, one-fifth of the minimum-lethal dose on eight occasions, then one-fourth, and one-third; and at longer intervals, the minimum-lethal, twice, four times, six times, eight times, ten times, and so on, until, on the 116th day, a dose eighty times larger than the minimum-lethal was introduced into the stomach.

No observable disturbance was produced by any of these doses.

As in further administrations, doses of upwards of a gramme of dry venom would have been required, the experiment was not continued beyond this point, for such large quantities would have soon exhausted the rapidly-diminishing supply of venom.

Eight days after the animal had received by the stomach a dose of venom representing eighty times the minimum-lethal if given subcutaneously, a dose of venom corresponding to one and a half times the minimum-lethal was injected under the skin. No obvious general symptoms followed the administration of this dose, but some local œdema and skin necrosis were produced, and the animal has remained in good health until the present time.

During this experiment, an opportunity occurred for obtaining other facts of some interest. It happened that when the administrations of venom were commenced, the animal was already pregnant, and on the 54th day of the experiment two healthy kittens were born. These kittens were fed exclusively on the mother's milk, the mother continuing to receive gradually increasing doses of venom.

One of the kittens, when fifty-seven days old, and when the mother had last received a dose equivalent to thirty times the minimum-lethal if given subcutaneously, received, by subcutaneous injection, twice the minimum-lethal dose of cobra venom; and only slight symptoms, consisting chiefly of drowsiness and loss of appetite, were produced, from which the kitten completely recovered in a few hours.

The second kitten, when sixty-nine days old, received, also by subcutaneous injection, thrice the minimum-lethal dose; but the protection produced through the mother's milk was insufficient to antagonise this large dose of venom, and death followed the administration.

Evidence in favour of the production of protection by stomach administration, as well as of the toxic feebleness of venom when given by this channel, has been obtained with white rats also. Single doses, corresponding to 10, 20, 40, 200, 300, 600, and 1000 times the minimum-lethal if given subcutaneously, were given by stomach administration to each of seven different white rats. Sleepiness and loss of appetite, lasting for a day or two, were the only effects produced even by the larger of these enormous quantities, and all the animals entirely recovered.

A further experiment was made on the white rat which had received 1000 times the minimum-lethal dose. Seven days after this dose had been administered, and when the animal was apparently in good health, twice the minimum-lethal dose was injected under the skin. Distinct though not serious toxic symptoms were produced, consisting of sleepiness, anorexia, and increase of salivary and bronchial secretion; but in less than twenty-four hours these symptoms had disappeared, and the animal was soon afterwards in a perfectly normal state.

It would, therefore, appear that although serpents' venom, even in enormous quantities, fails to produce any toxic effects when introduced into the stomach, it still confers upon the animal a certain and not inconsiderable degree of resistance against the toxic effects of subsequent lethal doses of venom. That it does so by causing an antidotal substance to be present in the blood is also manifest from the result of the experiment on the kitten, which had been fed with milk derived from a parent receiving venom by the stomach.

In circumstances which are no doubt exceptional, some of these results would admit of useful practical application.

They probably also offered an explanation of the protection apparently enjoyed by certain snake-charmers, as well as by individuals who claim to be protected, whether members of special sects or not; for subcutaneous injection is not likely to be the method, and it certainly was not the method several hundreds of years ago, employed for the introduction of the protection-producing venom into their bodies.

Antidotal Properties of the Blood-Serum of Venomous Serpents.—The results of these experiments may explain also the clearly-established protection possessed by venomous serpents themselves.

They, as well as other circumstances, render it important to determine whether the blood of venomous serpents contains, as does that of artificially-protected animals, an actual substance possessing antidotal qualities.

In order to arrive at some definite conclusion on this subject, I have made endeavours to obtain living venomous serpents, and also the serum separated from their blood.

Last year, an arrangement was concluded with one of the best known of the importers of wild animals to supply me with living cobras. He, however, has not succeeded in doing so, because of some exceptional difficulties; but, as an alternative, he has recently sent me several living specimens of the Hamadryas (*Ophiophagus elaps*), a serpent of greater size and more aggressive disposition than the cobra, and reputed to be at least as deadly as it.

A few days after their arrival, it was observed that moulting was about to commence; and as the condition of health is deteriorated during this process, blood has not yet been taken from any living Hamadryas. One of them, however, became sickly and died. A short time after its death, the neck blood-vessels were opened, and, as coagulation fortunately had not occurred, a small quantity of blood was collected, from which a little blood-serum afterwards separated. As no liquid venom could be obtained from this Hamadryas, this serum has been tested against cobra venom. Two experiments were made, in which it was mixed with slightly more than the minimum-lethal

dose of cobra venom, and the mixture then injected under the skin of rabbits. When the quantity of Hamadryas serum was ·15 c.c. per kilogramme of animal, death was not prevented; but as the animal did not die until more than seven hours, an antidotal effect had apparently been produced by this quantity of serum. In the second experiment, a larger quantity of serum was used—namely, ·25 c.c. per kilogramme, and the result was entirely successful; for not only did the animal survive, but no decided symptoms of poisoning were manifested during the six hours in which the animal remained under nearly continuous observation.

Two experiments were also made in which this antivenene was administered thirty minutes after rather more than the minimum-lethal dose of cobra venom. In the first, the dose of antivenene was ·3 c.c. per kilogramme; but this dose was found to be an insufficient one, for the animal died in four hours. In the second experiment, ·5 c.c. per kilogramme of antivenene was administered, in the same conditions as in the former experiment, and it proved to be a sufficient quantity, for the animal recovered, after manifesting only slight toxic symptoms.

I hope by-and-by to extend these observations with blood-serum and venom, taken in more favourable circumstances, from the other and larger Hamadryas, which are now apparently in a state of excellent health.

It has, however, already been possible to confirm these results with the blood-serum and venom of another species of serpent. Dr Thomas Bancroft, of Brisbane, Australia, has recently sent me the dried blood-serum of three black snakes (*Pseudechis porphyriacus*) of that country, and also some dried venom removed from the poison-glands of the same three serpents.

The venom, as it has reached me, is not a very active one, the minimum-lethal dose for rabbits being between ·003 and ·0035 gramme for each kilogramme of animal. At the same time, although this serpent is a member of the Colubrine family, the irritative effects at the position of injection, and even more so on the kidneys following its absorption, are intense. In all the experiments made with the venom alone, the urine voided within a few hours was of a dark red, almost black colour, and was found to contain a large quantity of hæmoglobin, but no blood-cells.

Although the quantity of dry serum was small, there was sufficient to allow three experiments to be made, for the purpose of determining if it can prevent death from being produced by a lethal dose of venom, when the two are mixed together before administration. In one of these experiments, the dose of serum was 1 c.c., and that of venom ·0035 gramme per kilogramme of animal; in the second, the dose of serum was ·5 c.c., and the dose of venom the same as in the first experiment; and in the third experiment, the dose of serum was 1 c.c., and that of venom ·004 gramme per kilogramme of animal. In each case, the gratifying result was obtained that the animal survived the administration of these lethal doses of venom.

It has thus been shown that venomous serpents themselves possess a definite substance in the blood-serum, which possesses antidotal properties against their own venom and the venom of other species of serpents.

It is probable that the substance is produced from venom shed upon the mouth-surface, and absorbed into the blood from this surface or elsewhere in the alimentary canal, and also from venom absorbed directly into the lymphatics and blood-vessels of the poison-glands. At the same time, the protection which is enjoyed by several species of serpents may also be produced by venom introduced into the body with the venomous snakes on which some of them, and especially the Hamadryas, largely subsist.

The blood-serums of the two species of venomous serpents that have been examined are certainly not so powerfully antivenene as the serum which can be obtained from artificially protected animals. They have, however, been obtained in conditions which are not the most favourable for determining the true value of the blood-serum of serpents. This can probably only be done in the countries in which the serpents are found.

If this natural antivenene be found to be powerful, then a new, and in some respects convenient, source for antivenene will become available; but even if the antidotal power be not so great as that of the serum of artificially protected animals, it is possible that its value may be increased, and a sufficiently powerful antidote obtained, more rapidly than with entirely unprotected animals, by injecting several successive doses of venom into the serpents themselves.